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(54) WF11243 SUBSTANCE

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(56) References cited:
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Description**FIELD OF THE INVENTION**

5 The present invention relates to WF11243 substance and its salts as well as to a method of preparing them and use of them. The WF11243 substance is a novel substance which is isolated and purified from a culture broth of microorganisms, especially fungi. It has excellent fungicidal and protozoicidal activity and is, therefore, useful as various preventing and treating medicines for preventing, curing and reducing various diseases and disorders to be caused by fungi and protozoans. It is also useful for prevention and treatment of Pneumocystis carinii pneumonia.

10 Therefore, the present invention plays an important role in various technical fields of medicines, cosmetics, foods and drinks.

PRIOR ART

15 Hitherto, various fungicides have been obtained from cultures of microorganisms or by chemical syntheses. Some of them could be admitted to be good, but many of them have various problems, such as appearance of drug-tolerant microorganisms and safety of themselves. At present, no one has a satisfactory fungicidal agent which is completely free from the problems.

20 Recently, various diseases to be caused by protozoans, especially those to be caused by Pneumocystis carinii such as Pneumocystis carinii pneumonia, have been closed up, and development of methods of preventing and treating them has been strongly desired in this technical field.

Japanese Patent Abstracts, page 78c 620, abstract of patent 1-104097 discloses similar fermentation products as mentioned below, which, however, do not have a residue of beta-oxypalmitic acid.

PROBLEMS TO BE SOLVED BY THE INVENTION

25 The present invention has been made in view of the current technical situation as mentioned above, and the object of the present invention is to provide and develop a novel protozoicidal agent which is effective for preventing the growth of Pneumocystis carinii which causes Pneumocystis carinii pneumonia and other diseases, or protozoans. Another object of this invention is to develop and provide novel fungicides useful for preventing the growth of various fungi.

MEANS TO SOLVE THE PROBLEMS

35 In order to attain the above-mentioned object, the present inventors investigated from various aspects and noted natural substances, especially fermented products from microorganisms, in view of safety. They studied and investigated various microorganisms and, as a result, have found that fungal strain No. 11243 as isolated from fallen leaves samples collected in Ayabe-shi, Kyoto-fu, Japan, produces and accumulates the intended product in the culture broth. They have further studied in detail the product with respect to the physico-chemical properties thereof and have found that the product is a novel substance. They have named the substance WF11243 substance. Further, they have named the hydrochloride thereof FR901469 substance. After further study of the novel WF11243 substance, they have established an industrial method of producing them and have achieved the present invention.

BRIEF EXPLANATION OF THE DRAWINGS

45 Fig. 1 shows a ^{13}C nuclear magnetic resonance spectrum of FR901469 substance.

Fig. 2 is ^1H nuclear magnetic resonance spectrum of FR901469 substance.

The WF11243 substance of the present invention is a novel substance having, as its hydrochloride, FR901469 substance, the physico-chemical properties as mentioned below.

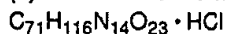
Physico-chemical Properties of FR901469 Substance (1):

(1) Appearance:
white powder

55 (2) Melting point:
182 to 187°C

(3) Specific rotation:
 $[\alpha]_D^{23} +29^\circ$ (c=1.5, methanol)

(4) Molecular formula:



(5) Element analysis:

Calcd (%) for $C_{71}H_{116}N_{14}O_{23} \cdot HCl \cdot 8H_2O$			
	C 49.74,	H 7.82,	N 11.44
Found (%):	C 49.65,	H 7.72,	N 11.40

(6) Solubility:

soluble: methanol, water

slightly soluble: acetone

insoluble: n-hexane

(7) Color reaction:

positive: iodine vapor reaction, ceric sulfate reaction, ninhydrin reaction

negative: Molish reaction, Ehrlich reaction

(8) Thin layer chromatography (TLC):

Stationary phase	Developing solvent	Rf value
Silica gel 60 F ₂₅₄ (made by E. Merck)	n-butanol/acetic acid/water (4/1/2)	0.42
RP-18 WF ₂₅₄ S (made by E. Merck)	45% aqueous acetonitrile (containing 0.5% $NH_4H_2PO_4$)	0.18

(9) Infrared absorption spectrum (FT-IR (KBr)): Significant peaks are:

 ν_{\max}^{KBr} : 3400, 2920, 1730, 1660, 1650, 1635, 1540, 1520, 1460, 1250, 1090 cm^{-1}

(10) High performance liquid chromatography (HPLC):

column: YMC • Pack ODS-AM (AM-303, S-5, 120A, 4.6 mm ID × 250 mm; YMC Co., Ltd.)

detection: 210 nm

developing solvent: 45% aqueous acetonitrile (containing 0.5% $NH_4H_2PO_4$)

flow rate: 1 ml/min

retention time (RT): 10.9 min

(11) ^{13}C Nuclear magnetic resonance spectrum (100 MHz, CD_3OD):

Fig. 1 shows the chart.

δ_c : 176.1 (s), 174.6 (s), 174.0 (s), 173.8 (s), 173.4 (s), 172.8 (s), 172.6 (s), 172.5 (s), 172.4 (s), 172.1 (s), 171.9 (s), 171.8 (s), 171.7 (s), 171.2 (s), 156.1 (s), 131.4 (d) ×2, 128.6 (s), 117.9 (d) ×2, 74.2 (d), 72.0 (d), 70.5 (d), 70.1 (d), 70.0 (d), 69.1 (d), 68.8 (d), 68.3 (d), 67.7 (d), 61.4 (d), 60.9 (d), 60.3 (d), 59.8 (d), 58.2 (d), 57.9 (t), 57.6 (d), 57.2 (d), 56.8 (d), 51.7 (d), 50.8 (d), 46.4 (t), 43.1 (t), 42.9 (t), 40.4 (t), 39.9 (t), 38.9 (t), 37.6 (t), 35.8 (t), 34.7 (t), 33.1 (t), 31.6 (d), 30.8 (t), 30.7 (t), 30.7 (t), 30.7 (t), 30.6 (t), 30.5 (t), 30.4 (t), 30.3 (t), 28.1 (t), 25.9 (t), 24.9 (t), 23.7 (t), 21.2 (q), 20.9 (q), 20.3 (q), 19.2 (q), 18.9 (q), 18.7 (q), 17.2 (q), 14.4 (q).

(12) ^1H Nuclear magnetic resonance spectrum (400 MHz, CD_3OD):

Fig. 2 shows the chart.

δH : 7.08 (2H, d, $J=8\text{Hz}$), 6.98 (2H, d, $J=8\text{Hz}$), 5.18-5.09 (2H, m), 4.88 (1H, br s), 4.76-4.69 (3H, m), 4.65 (1H, m), 4.48 (1H, m), 4.46 (1H, d, $J=5\text{Hz}$), 4.41 (1H, d, $J=9\text{Hz}$), 4.36-4.27 (3H, m), 4.24-4.13 (6H, m), 3.98 (1H, m), 3.92-3.78 (3H, m), 3.70 (1H, dd, $J=11$ and 4Hz), 3.58 (1H, br d, $J=11\text{Hz}$), 3.00-2.93 (3H, m), 2.80 (1H, dd, $J=13$ and 11Hz), 2.56 (1H, dd, $J=13$ and 11Hz), 2.45 (1H, dd, $J=14$ and 9Hz), 2.37 (1H, br d, $J=13\text{Hz}$), 2.30 (1H, m), 2.21-2.05 (3H, m), 2.03-1.93 (2H, m), 1.89 (1H, m), 1.77 (1H, m), 1.62-1.50 (4H, m), 1.39 (3H, d, $J=6\text{Hz}$), 1.35 (3H, d, $J=6.5\text{Hz}$), 1.35 (3H, d, $J=6.5\text{Hz}$), 1.30 (3H, d, $J=6\text{Hz}$), 1.28 (22H, m), 1.13 (3H, d, $J=6\text{Hz}$), 0.89 (3H, t, $J=7\text{Hz}$), 0.81 (3H, d, $J=6.5\text{Hz}$), 0.80 (3H, d, $J=6.5\text{Hz}$),

(13) Molecular formula and FAB-MS:

$\text{C}_{71}\text{H}_{116}\text{N}_{14}\text{O}_{23} \cdot \text{HCl}$

FAB-MS m/z 1533 ($\text{M}+\text{H}$) $^+$;

HRFAB-MS m/z 1555.8240

(calcd for $\text{C}_{71}\text{H}_{116}\text{N}_{14}\text{O}_{23}\text{Na}$: 1555.8235)

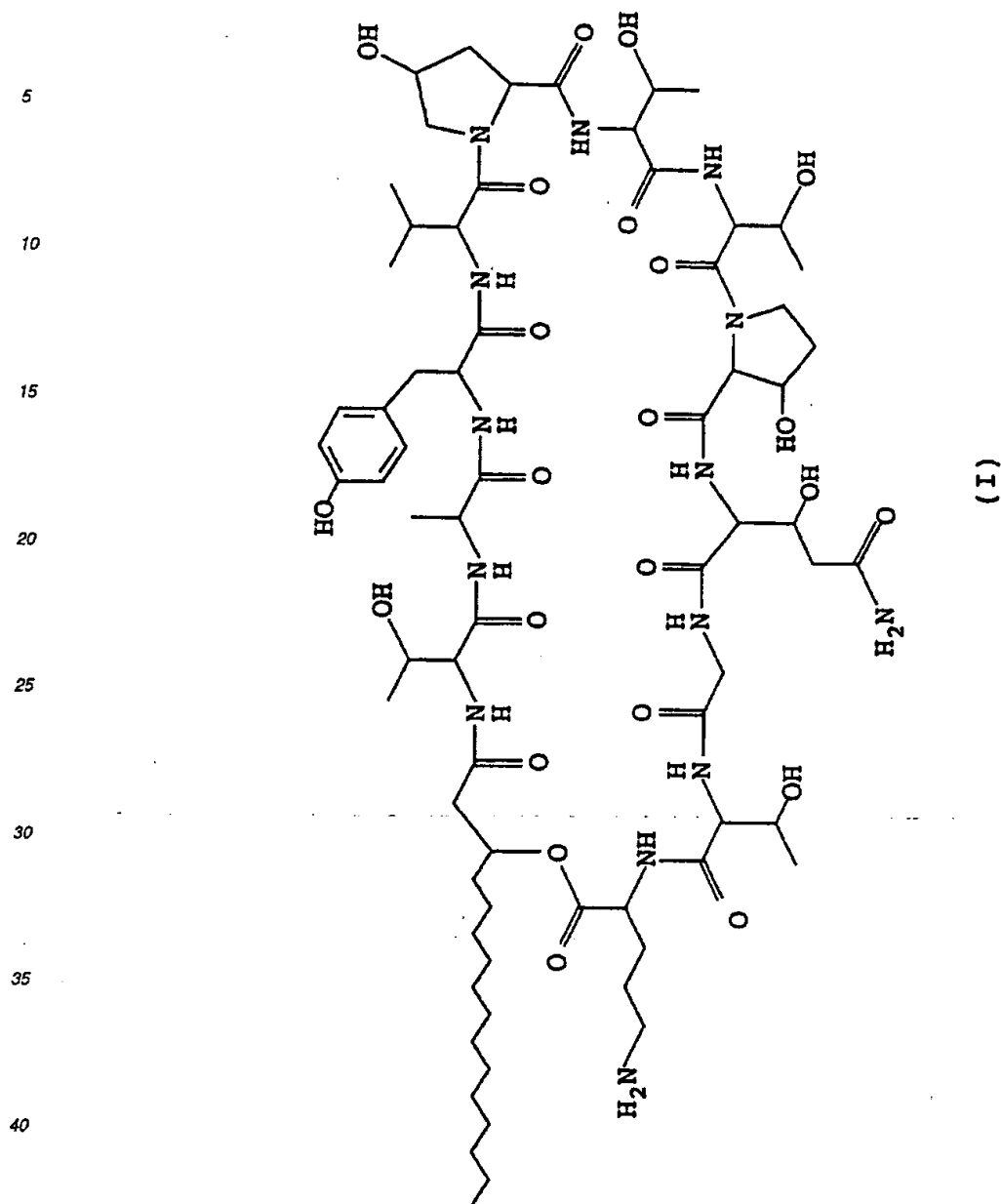
(14) Analysis of amino acids:

Thr (4), Gly (1), Ala (1), Val (1), Tyr (1), Orn (1) (as hydrolyzed in 6 N HCl at 110°C for 20 hours)

(15) Ultraviolet absorption spectrum: Significant peaks are:

$\lambda_{\text{max}}^{\text{H}_2\text{O}}$: 225 (sh), 275 (1200), 280 (sh) nm
 $\lambda_{\text{max}}^{0.01\text{N HCl}}$: 225 (sh), 275, 280 (sh) nm
 $\lambda_{\text{max}}^{0.01\text{N NaOH}}$: 240, 292 nm

From the above-mentioned physico-chemical properties, the WF11243 substance of the present invention was recognized to have polypeptide-like characteristics. As a result of an attempt to determine the structure of the substance, the present inventors have succeeded in the elucidation to obtain the following presumed structural formula (I) for the substance:



The WF11243 substance of the present invention is produced, for example, by the fungus strain No. 11243 isolated from fallen leaves collected by the present inventors in Ayabe-shi, Kyoto-fu, and may be also produced by chemical syntheses such as peptide synthesis. This organism grew restrictedly on various culture media, and formed pale orange colonies. Its mycological characteristics were as follows.

Cultural characteristics on various agar media are summarized in Table 1. After innoculating at the center on a malt extract agar medium, the culture grew very restrictedly attaining 0.5 to 1.0 cm in diameter after two weeks at 25°C. This colony surface was raised and pale orange and it produced hyaline mucous exudation. The reverse was grayish orange. Conidial structures were observed. Colonies on potato dextrose agar grew very restrictedly, attaining 0.5 to 1.0 cm in diameter, under the same condition. The surface was raised, radially sulcate and pale orange and it produced hyaline mucous exudation. The reverse was pale yellow. Anamorphosis (conidial structures) formed.

Table 1

Medium	Cultural characteristics of the Strain No. 11243
Malt extract agar	G: Very restrictedly, 0.5 to 1.0 cm in diameter S: Circular, raised, formed conidial structures, produced hyaline mucous exudation, pale orange (5A3) R: Grayish orange (5B4)
Potato dextrose agar	G: Very restrictedly, 0.5 to 1.0 cm in diameter S: Circular, raised, radially sulcate, formed conidial structures, produced hyaline mucous exudation, pale orange (5A3) R: Pale Yellow (4A3)
Czapek-Doc agar	G: Very restrictedly, 0.1 to 0.5 cm in diameter S: Circular, plane, thin, formed conidial structures, orange white (5A2) R: Yellowish gray (4B2)
Sabouraud agar	G: Very restrictedly, 0.5 to 1.0 cm in diameter S: Circular, raised, radially sulcate, formed no conidial structures, produced hyaline mucous exudation, grayish orange (5B3) R: Brown (6E7)
Oatmeal agar	G: Very restrictedly, 0.5 to 1.0 cm in diameter S: Circular to irregular, radially sulcate, formed conidial structures, orange white (5A2) black at center R: Pale Yellow (4A3)
Yp Ss agar	G: Very restrictedly, 0.5 to 1.0 cm in diameter S: Circular, raised, radially sulcate, formed no conidial structures, produced hyaline mucous exudation, light orange (5A4) R: Pale Yellow (4A3)
Corn meal agar	G: Very restrictedly, 0.5 to 1.0 cm in diameter S: Circular to irregular, radially sulcate, formed conidial structures, produced hyaline mucous exudation, orange white (5A2), dark brown (6F8) at center R: Dark Gray (1F1)
MY20 agar	G: Very restrictedly, 0.1 to 0.5 cm in diameter S: Irregular, raised, wrinkly, formed conidial structures, produced hyaline mucous exudation, light orange (5A4) R: Light orange (5A4)
Abbreviation G: growth S: colony surface R: reverse	

These characteristics were observed after 14 days of incubation at 25°C. The color description were based on the Methuen Handbook of Colour⁽¹⁾. (1) Kornerup, A. and J.H. Wanscher: Methuen Handbook of Colour (3rd ed.), Methuen, London, 1983

The No. 11243 strain may grow at 7 to 29°C, and the optimum growth temperature for the strain is 22 to 26°C (as

measured on a potato dextrose agar).

Totally considering these characteristics, we identified that the No. 11243 strain belongs to the group of fungi and named it No. 11243. This was deposited with the National Institute of Bioscience and Human Technology, Agency of Industrial Science and Technology, Ministry of International Trade and Industry (Deposit No.: FERM BP-3373; Deposit date: April 23, 1991).

It should be understood that production of the WF11243 substance is not restricted to only the use of the particular microorganism which has been referred to herein only for explanation of producing the substance. The present invention includes use of all artificial and natural variants and mutants capable of producing the WF11243 substance, which may be obtained by variation or mutation of the microorganism of the described strain by X-ray irradiation or ultraviolet irradiation or by treatment with N-methyl-N'-nitro-N-nitrosoguanidine, 2-aminopurine or the like.

The WF11243 substance of the present invention can be produced by inoculating fungi capable of producing the substance (for example, strain No. 11243) in a nutrient medium containing assimilable carbon and nitrogen sources and incubating them therein under an aerobic condition (for example, by shaking culture or submerged aerobic culture).

As usable carbon sources, preferred are glucose, sucrose, starch, modified starch, fructose, glycerin and other hydrocarbons.

As usable nitrogen sources, preferred are oatmeal, yeast extract, peptone, gluten meal, cotton seed flour, cotton seed refuse, soybean meal, corn steep liquor, dried yeast, wheat germ, peanut meal, chicken bone meal, etc. In addition, also advantageously usable are inorganic and organic nitrogen compounds, such as ammonium salts (for example, ammonium nitrate, ammonium sulfate, ammonium phosphate, etc.), ureas and amino acids.

These carbon sources and nitrogen sources are advantageously employed in combination, but they are not always needed to be pure. This is because some impure sources of these kinds would often contain growth factors or micro nutrients, advantageously.

If desired, inorganic salts, for example, those mentioned below could be added to the medium. Such salts include sodium carbonate, potassium carbonate, sodium phosphate, potassium phosphate, sodium chloride, potassium chloride, sodium iodide, potassium iodide, magnesium salts, copper salts, cobalt salts, etc.

If the medium used foams noticeably, liquid paraffin, animal oil, vegetable oil, mineral oil, silicone or the like may be added thereto, as the case may be.

Where industrial mass-production of a large amount of the intended product is desired, submerged aerobic culture of the microorganisms is recommended, like the case of producing other fermented products. Where production of a small amount of it is desired, shaking culture using a flask is preferred.

Where the culture of the microorganisms of producing the intended WF11243 substance is effected in a large tank, it is desired that the microorganisms are first inoculated and incubated in a relatively small amount of a medium and then the resulting culture is transferred to a large-sized production tank to further incubate it therein, for the purpose of preventing retardation of the growth of the cultivated microorganisms in the step of producing the intended WF11243 substance. In the case, the composition of the medium to be used for the pre-culture may be same as or different from, if desired, that of the medium to be used for the production culture.

Culture of the microorganisms is preferably effected under an aerobic stirring condition, to which any and every known means may be applied. For instance, employable is stirring with propellers or other machines, or rotation or shaking of fermenters, or pumping, or air blowing. The air to be used for aeration is a sterilized one.

The incubation temperature may suitably be varied within the range at which the WF11243 substance-producing microorganisms of the present invention may produce the intended substance of the invention. In general, it is from 1 to 40°C, preferably from 14 to 36°C. The culture time varies, depending upon the culture condition and the amount of the microorganisms to be inoculated. In general, it is approximately from one day to one week.

After fermentation, the intended WF11243 substance is collected from the culture broth. Briefly, the cultured fungi are directly extracted with water and/or organic solvent or, after broken by mechanical, ultrasonic or the like known means, extracted with water and/or organic solvents. Then, WF11243 substance is isolated and purified by ordinary methods. In the case of the culture broth, WF11243 substance is recovered directly and then purified by ordinary methods.

As isolating and purifying methods, for example, suitable are a solvent extraction method with water, organic solvent or mixed solvents of them, a chromatographic method, and a recrystallization method from single solvents or mixed solvents. Such ordinary methods may be combined.

Isolation and purification of the WF11243 substance produced may suitably be effected by means of the above-mentioned known methods. The substance may be isolated and purified, for example, by the following method. Briefly, the culture is extracted with an aqueous acetone, adsorbed to a neutral adsorbing resin (for examples, HP-20, made by Mitsubishi Kasei Corp., Japan) under a neutral condition, eluted with an acidified aqueous acetone, concentrated, washed with ethyl acetate, and extracted with butanol. If desired, the adsorption-desorption with the neutral adsorbing resin may be repeated. Thus, the intended product is collected as a pure form. The WF11243 substance is an amphoteric substance and therefore may react with a base or an acid to form a salt. The WF11243 substance may be recovered and purified even as its free form (WF11243 substance of itself) or may also be recovered and purified as its salt.

They (free form, salt) may be converted to each other by any known methods.

As salts of the WF11243 substance with bases, there are mentioned alkali metal salts such as sodium salt or potassium salts, and alkaline earth metal salts such as calcium salt or magnesium salt, and salts with inorganic bases such as ammonium salt, and salts with organic bases, such as organic amine salts including methylamine salt, ethylamine salt, propylamine salt, iso-propylamine salt, butylamine salt, t-butylamine salt, dimethylamine salt, diethylamine salt, trimethylamine salt, triethylamine salt, pyridine salt, picoline salt, dicyclohexylamine salt, N,N'-dibenzylethylenediamine salt and amino acid salts including arginine salt, asparagine salt and glutamic acid salt. As salts thereof with acids, there are exemplified inorganic acid-addition salts such as hydrochloric acid salt, hydrobromic acid salt, hydroiodic acid salt, sulfuric acid salt, nitric acid salt and phosphoric acid salt, organic acid-addition salts such as acetic acid salt, trifluoroacetic acid salt, maleic acid salt, tartaric acid salt, methanesulfonic acid salt, benzenesulfonic acid salt, formic acid salt and toluenesulfonic acid salt, and amino acid-addition salts such as aspartic acid salt and glutamic acid salt, etc.

The present invention also provides a pharmaceutical composition containing the WF11243 substance and/or its salt as an active ingredient. It can be used in the form of a pharmaceutical preparation, for example, in solid, semisolid or liquid form, which contains the WF11243 substance and/or its salt, as an active ingredient, in admixture with an organic or inorganic carrier or excipient suitable for rectal, pulmonary (nasal or buccal inhalation), nasal, ocular, external (topical), oral or parenteral (including subcutaneous, intravenous and intramuscular) administrations or insufflation.

The active ingredient may be compounded, for example, with ordinary non-toxic, pharmaceutically acceptable carriers for tablets, pellets, troches, capsules, suppositories, creams, ointments, aerosols, powders for insufflation, solutions, emulsions, suspensions, and any other form suitable for use. And, if necessary, in addition, auxiliary, stabilizing, thickening and coloring agents and flavours may be used.

The WF11243 substance and/or its salt are/is included in the pharmaceutical composition in an amount sufficient to produce the desired effect to the process or status of diseases.

For applying the composition to human beings, it is preferable to apply it by intravenous, intramuscular, or oral administration. While the dosage of the therapeutically effective amount of the active ingredient varies and depends upon the age and condition of each individual patient to be treated; in the case of intravenous administration, a daily dose of from 0.01 to 100 mg of the active ingredient per kg weight of a human being, in the case of intramuscular administration, a daily dose of from 0.1 to 100 mg of the active ingredient per kg weight of a human being, in case of oral administration, a daily dose of from 0.5 to 100 mg of the active ingredient per kg weight of a human being is generally given for prevention or treatment of infectious diseases in human beings.

The WF11243 substance and/or its salts of the present invention are/is widely effective for prevention and/or treatment of various fungal infectious diseases and they are also effective for prevention and/or treatment of other various protozoiasis to be caused by various protozoans. In particular, they are effective for prevention and/or treatment of various Pneumocystis carinii infections (e.g., Pneumocystis carinii pneumonia). In use of the WF11243 substance and/or its salts of the present invention for prevention and/or treatment of Pneumocystis carinii pneumonia, the following points are to be noted.

For administration by inhalation, the compounds of the present invention are conveniently delivered in the form of an aerosol spray presentation from pressurized packs or nebulisers. The compounds may also be delivered as powders which may be formulated and the powder compositions may be inhaled with the aid of an insufflation powder inhaler device. The preferred delivery system for inhalation is a metered dose inhalation aerosol, which may be formulated as a suspension or solution of the compound in suitable propellants such as fluorocarbons or hydrocarbons.

Because of desirability to directly treat lung and bronchi, aerosol administration is a preferred method of administration. Insufflation is also a desirable method, especially where infection may have spread to ears and other body cavities.

Alternatively, parenteral administration may be employed using drip intravenous administration.

The present invention will be explained in more detail by way of the following examples.

Example 1:

(1) Fermenting Production of WF11243 Substance:

160 ml of a pre-culture medium comprising 4% of sucrose, 2% of cotton seed oil refuse, 1% of dried yeast, 1% of peptone, 0.2% of KH_2PO_4 , 0.2% of CaCO_3 and 0.1% of Tween 80 was put into each 500 ml-Erlenmeyer flask and sterilized at 121°C for 30 minutes. One loop of a slant-culture of fungal strain No. 11243 (FERM BP-3373) was inoculated to each medium and incubated at 25°C for 4 days by shaking cultivation.

Next, a fermenting medium comprising 2% of modified starch, 0.5% of glucose, 1% of cotton seed oil refuse, 1% of gluten meal, 2% of KH_2PO_4 , 1.5% of $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$, 0.001% of $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.025% of Adekanol LG-109 (made by Asahi Denka Co., Japan) and 0.025% of Silicone KM70 (made by Shin-etsu Chemical Co., Japan) was prepared, and 20 liters of the medium was put in a 30-liter jar fermenter. This was sterilized at 121°C for 30 minutes, and 2% of the previously obtained pre-culture was inoculated thereto and incubated at 25°C for 4 days. Stirring of the

medium was effected at 200 rpm and the amount of air introduced into the fermenter was 20 liter/min, during culture. The amount of the WF11243 substance as produced in the culture was determined by HPLC (column: Hibar Lichrosuper 100 RP 18, made by E. Merck; solvent: 50% aqueous acetonitrile containing 0.5% of $\text{NH}_4\text{H}_2\text{PO}_4$; detection: UV 210 nm; flow rate: 1 ml/min). The same amount of acetone was added to the liquid culture, the resulting mix was filtered, and the filtrate was concentrated to a determined concentration. The resulting concentrate was used as a sample for determination.

(2) Extraction and Purification of WF11243 Substance:

The same amount of acetone was added to 75 liters of the culture broth as obtained by the above-mentioned cultivation method and left at room temperature overnight with intermittently stirring the mix. This was filtered to obtain a culture extract. 65 liters of water was added to the liquid extract, followed by adjustment to have pH 6.5 with 6 N NaOH. This was then applied to 6.5 liters of HP-20 column (made by Mitsubishi Kasei Corp., Japan). After the column was washed with 35 liters of water and 27 liters of 40% aqueous acetone, and the intended product was eluted with 48 liters of 80% aqueous acetone containing, as a final concentration, 0.002 N HCl.

Purification was effected with on the basis of the fungicidal activity to Candida albicans as well as by HPLC (column: YMC Packed Column AM-303 (S-5, 120 A, ODS), made by YMC Co., Ltd.; mobile phase: 45% aqueous acetonitrile containing 0.5% $\text{NH}_4\text{H}_2\text{PO}_4$; detection: UV 210 nm; flow rate: 1 ml/min; retention time (RT): 10.9 min).

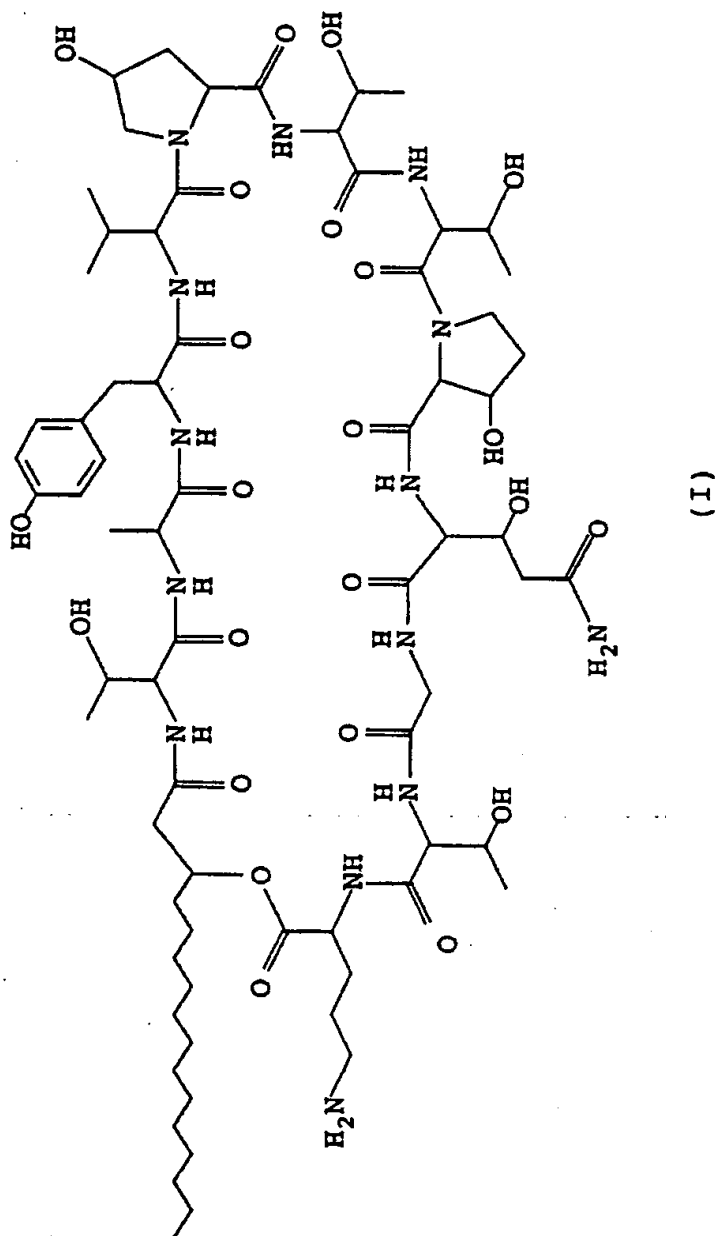
The eluate as obtained in the above was concentrated to 1.9 liters under reduced pressure, and it was adjusted to have pH of 6.0 and then washed with a double amount of ethyl acetate. Next, the active component was extracted out with 1.9 liters of butanol, and this was concentrated under reduced pressure and substituted by one liter of water. The resulting aqueous solution was adjusted to have pH 3.0 with 1 N HCl and again washed with one liter of ethyl acetate, then it was extracted with one liter of butanol to isolate the active component therefrom. The organic solvent layer was washed with one liter of 1% sodium bicarbonate and one liter of aqueous hydrochloric acid having pH 4, then the organic solvent layer was concentrated under reduced pressure. Next, the residue was dissolved in 3.0 liters of 50% aqueous acetonitrile and applied to a 1.3 liter HP-20 column. The column was washed with 3.5 liters of water, 3.5 liters of 50% aqueous methanol, 3.5 liters of 80% aqueous methanol and 3.8 liters of methanol, and the active component was eluted out therefrom with 2.7 liters of 80% aqueous acetone containing, as a final concentration, 0.002 N HCl. Next, the resulting eluate was concentrated under reduced pressure, the residue was dissolved in 5 liters of 20% aqueous methanol, and the solution was applied to a 500 ml-column of a reversed phase carrier (YMC Gel, ODS-AM, 120-S50, made by YMC Co., Ltd.). The column had previously been equilibrated with 20% aqueous acetonitrile containing 0.5% $\text{NH}_4\text{H}_2\text{PO}_4$. After the solution containing the intended product was charged in the column, the column was washed with one liter of 30% aqueous acetonitrile containing 0.5% $\text{NH}_4\text{H}_2\text{PO}_4$, one liter of 35% aqueous acetonitrile containing 0.5% $\text{NH}_4\text{H}_2\text{PO}_4$, and one liter of 40% aqueous acetonitrile containing 0.5% $\text{NH}_4\text{H}_2\text{PO}_4$. The intended product was eluted out with two liters of 45% aqueous acetonitrile containing 0.5% $\text{NH}_4\text{H}_2\text{PO}_4$.

280 ml of the thus eluted active fraction was diluted with the same amount of water and again applied to a 180 ml-column of a reversed phase carrier (YMC Gel, ODS-AM, 120-S50). After the column was washed with 0.4 liter of 30% aqueous acetonitrile containing 0.5% $\text{NH}_4\text{H}_2\text{PO}_4$, 0.4 liter of 35% aqueous acetonitrile containing 0.5% $\text{NH}_4\text{H}_2\text{PO}_4$ and 0.4 liter of 40% aqueous acetonitrile containing 0.5% $\text{NH}_4\text{H}_2\text{PO}_4$, it was developed with 43% aqueous acetonitrile containing 0.5% $\text{NH}_4\text{H}_2\text{PO}_4$ to elute the intended substance. 55 ml of the eluted active fraction was diluted with the same amount of water and then applied to a 40 ml-column of HP-20.

The column was washed with 180 ml of water and the intended product was eluted with 100 ml of 80% aqueous acetone containing, as a final concentration, 0.002 N HCl. The resulting eluate was concentrated under reduced pressure and, after acetone was removed, freeze-dried to obtain 72 mg of a white powder of hydrochloride of WF11243 substance. This is called FR901469 substance.

(3) Physico-chemical Properties of FR901469 Substance:

Physico-chemical properties of FR901469 substance thus obtained are previously shown. Analysis of amino acids was effected in the manner as mentioned below. One mg of FR901469 substance was hydrolyzed with one ml of 6 N HCl in a sealed tube at 110°C for 20 hours. After hydrolysis, the reaction mixture was evaporated to dryness, and the resulting solid was analyzed with an amino acid analyzer (Hitachi 835 Automatic Amino-Acid Analyzer). The result indicated that the product comprised Thr (4), Gly (1), Ala (1), Val (1), Tyr (1), and Orn (1). The chemical structure of the product was determined to be as follows:



Example 2: Biological Properties of FR901469 Substance

(1) Microbicidal Activity:

The microbicidal activity of the FR901469 substance was measured by an ordinary microbroth dilution method using a 96-well multi-tray, which is mentioned below.

Microorganisms to be tested (mentioned below) were incubated on a slant medium. From the thus incubated cells, a test cell suspension (containing 2×10^5 living cells per ml) was prepared with an yeast nitrogen base dextrose (YNBD) medium. A continuous 1/2 diluted series of the FR901469 substance in YNBD was prepared and 100 μ l was put in each well, and further 100 μ l of the test cell suspension was added thereto and incubated therein at 37°C for 24 hours (for *Candida* and *Aspergillus*), or for 48 hours (for *Cryptococcus*). After incubation, the turbidity of each well was measured, and the concentration of the active substance indicating a half (1/2) of the turbidity of the control well (not containing the

active substance) was represented as the 50% growth inhibiting concentration (IC_{50}). The results obtained are shown in Table 2 below.

Table 2

Microbicidal Activity of FR901469 Substance	
Tested Microorganisms	IC_{50} (μ g/ml)
Candida albicans	FP582 <0.0093
	FP578 0.16
	FP629 0.16
	FP633 0.16
Candida utilis	YC123 0.16
Candida krusei	YC109 0.0039
Candida tropicalis	YC118 0.0039
Cryptococcus neoformans	YC203 >10
Aspergillus fumigatus	8004 <0.0093
Aspergillus niger	ATCC9642 0.63

(2) Preventing effect of FR901469 substance on a mouse Candida albicans infection:

The in vivo effectiveness of FR901469 substance as a fungicide was demonstrated by the experiment mentioned below.

4-week ICR female mice (body weight: 18 to 21 g) were used as test animals. One test group comprised five mice. A suspension of living cells of Candida albicans FP633 as suspended in a physiological saline solution was injected to the vein of the tail of each mice in an amount of 2×10^6 living cells per mouse, whereby all mice were infected with the fungi. One hour after infection, an aqueous physiological saline solution of FR901469 substance was subcutaneously injected to each mouse. The subcutaneous injection was repeated once a day for continuous 3 days from the next day of infection. On Day 14 after infection, the number of the living mice was counted. The results obtained are shown in Table 3 below.

Table 3

<u>In vivo</u> preventing effect of FR901469 substance on infection	
Dose (mg/kg) of FR901469 Substance	Number of Living Mice
10	5
1	5
0	0

(3) Toxicity Test:

100 mg/kg of FR901469 substance was intraperitoneally injected to each of five 5-week ICR female mice, once a day, for continuous 3 days. As a result, no mice died. The body weight increase of all the test mice was same as that of the mice of a control group to which the substance was not injected. Thus, the high safety of the WF11243 substance was verified.

Example 3: Preparation of Injection

(1) FR901469 substance prepared in Example 1	5 g
(2) Edible salt (NaCl)	9 g
(3) Sodium hydrogencarbonate	1 g

All the components (1) to (3) were dissolved in 100 ml of distilled water and 1 ml was put into each ampule to prepare 1000 ampules for injection.

Example 4: Preparation of Aerosol for Inhalation:

2% by weight of the compound of formula (I), 33% by weight of ethanol, and 65% by weight of a propellant (mixture of propane/isobutane = 70/30) were charged into a valve-equipped pressure container to prepare an aerosol for inhalation.

EFFECT OF THE INVENTION

The present invention provides WF11243 substance, which is an unknown novel physiologically active substance. It displays excellent fungicidal and protozoicidal activity and is extremely useful for inhibition of the growth of fungi and protozoans and also for killing them in various technical fields of medicines, cosmetics, industrial drugs and, foods and drinks. The substance of the present invention is characterized in that it has both fungicidal activity and protozoicidal activity. Regarding the latter activity, for example, the substance is also characterized by the outstanding effectiveness for prevention and treatment of *Pneumocystis carinii* pneumonia.

In addition, since the structure of the active ingredient of the present invention has been presumed, industrial production of the substance by organic synthesis may be possible, as well as industrial production using microorganisms. As a result, production of various derivatives of the substance may be possible. Under the situation, production of many novel compounds and development of new uses of them can be expected much.

References to the microorganism deposited under Rule 13.2

1. No. 11243

(a) Name and Address of the depository with which said microorganism is deposited.

Name: National Institute of Bioscience and Human Technology, Agency of Industrial Science and Technology,
Ministry of International Trade and Industry

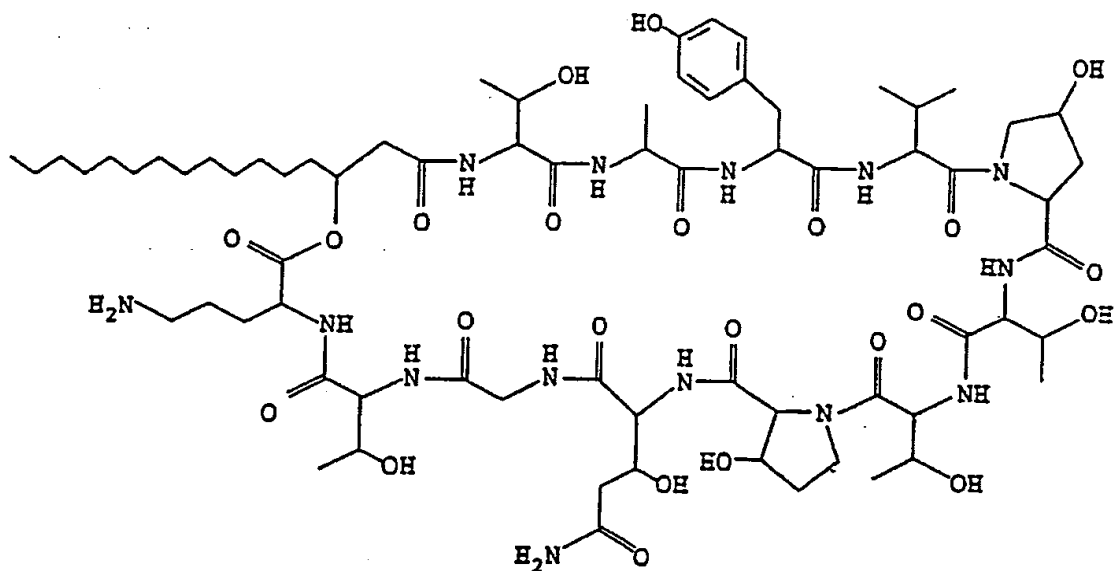
Address: 1-3, Higashi 1 chome, Tsukuba-shi, Ibaraki-ken, 305, Japan

(b) Date on which the deposition was made: April 23, 1991

(c) Deposit No. (Accession No.) assigned to the deposit by the depository (a): FERM BP-3373

Claims

1. WF11243 substance having the following chemical formula (I) or its salts.



(I)

2. WF11243 substance having, as its hydrochloride, the following physico-chemical properties or its salts:

(1) Appearance:
white powder

(2) Melting point:
182 to 187°C

(3) Specific rotation:
[α]_D²³ +29° (c=1.5, methanol)

(4) Molecular formula:
C₇₁H₁₁₆N₁₄O₂₃ · HCl

(5) Elemental analysis:

Calcd (%): for C ₇₁ H ₁₁₆ N ₁₄ O ₂₃ · HCl · 8H ₂ O			
	C 49.74,	H 7.82,	N 11.44
Found (%):	C 49.65,	H 7.72,	N 11.40

(6) Solubility:

soluble: methanol, water
slightly soluble: acetone
insoluble: n-hexane

(7) Color reaction:

positive: iodine vapor reaction, ceric sulfate reaction, ninhydrin reaction

negative: Molish reaction, Ehrlich reaction

(8) Thin layer chromatography (TLC):

Stationary phase	Developing solvent	Rf value
Silica gel 60 F ₂₅₄ (made by E. Merck)	n-butanol/acetic acid/water (4/1/2)	0.42
RP-18 WF ₂₅₄ S (made by E. Merck)	45% aqueous acetonitrile (containing 0.5% NH ₄ H ₂ PO ₄)	0.18

(9) Infrared absorption spectrum (FT-IR (KBr)): Significant peaks are

 $\nu_{\text{max}}^{\text{KBr}}$: 3400, 2920, 1730, 1660, 1650, 1635, 1540, 1520, 1460, 1250, 1090 cm⁻¹

(10) High performance liquid chromatography (HPLC):

column: YMC • Pack ODS-AM (AM-303, S-5, 120A, 4.6 mm ID x 250 mm; YMC Co., Ltd.)

detection: 210 nm

developing solvent: 45% aqueous acetonitrile (containing 0.5% NH₄H₂PO₄)

flow rate: 1 ml/min

retention time (RT): 10.9 min

(11) ¹³C Nuclear magnetic resonance spectrum (100 MHz, CD₃OD):

Fig. 1 shows the chart.

δ_{C} : 176.1 (s), 174.6 (s), 174.0 (s), 173.8 (s), 173.4 (s), 172.8 (s), 172.6 (s), 172.5 (s), 172.4 (s), 172.1 (s), 171.9 (s), 171.8 (s), 171.7 (s), 171.2 (s), 156.1 (s), 131.4 (d) x2, 128.6 (s), 117.9 (d) x2, 74.2 (d), 72.0 (d), 70.5 (d), 70.1 (d), 70.0 (d), 69.1 (d), 68.8 (d), 68.3 (d), 67.7 (d), 61.4 (d), 60.9 (d), 60.3 (d), 59.8 (d), 58.2 (d), 57.9 (t), 57.6 (d), 57.2 (d), 56.8 (d), 51.7 (d), 50.8 (d), 46.4 (t), 43.1 (t), 42.9 (t), 40.4 (t), 39.9 (t), 38.9 (t), 37.6 (t), 35.8 (t), 34.7 (t), 33.1 (t), 31.6 (d), 30.8 (t), 30.7 (t), 30.7 (t), 30.7 (t), 30.6 (t), 30.5 (t), 30.4 (t), 30.3 (t), 28.1 (t), 25.9 (t), 24.9 (t), 23.7 (t), 21.2 (q), 20.9 (q), 20.3 (q), 19.2 (q), 18.9 (q), 18.7 (q), 17.2 (q), 14.4 (q).

(12) ¹H Nuclear magnetic resonance spectrum (400 MHz, CD₃OD):

Fig. 2 shows the chart.

δ_{H} : 7.08 (2H, d, J=8Hz), 6.98 (2H, d, J=8Hz), 5.18-5.09 (2H, m), 4.88 (1H, br s), 4.76-4.69 (3H, m), 4.65 (1H, m), 4.48 (1H, m), 4.46 (1H, d, J=5Hz), 4.41 (1H, d, J=9Hz), 4.36-4.27 (3H, m), 4.24-4.13 (6H, m), 3.98 (1H, m), 3.92-3.78 (3H, m), 3.70 (1H, dd, J=11 and 4Hz), 3.58 (1H, br d, J=11Hz), 3.00-2.93 (3H, m), 2.80 (1H, dd, J=13 and 11Hz), 2.56 (1H, dd, J=13 and 11Hz), 2.45 (1H, dd, J=14 and 9Hz), 2.37 (1H, br d, J=13Hz), 2.30 (1H, m), 2.21-2.05 (3H, m), 2.03-1.93 (2H, m), 1.89 (1H, m), 1.77 (1H, m), 1.62-1.50 (4H, m), 1.39 (3H, d, J=6Hz), 1.35 (3H, d, J=6.5Hz), 1.35 (3H, d, J=6.5Hz), 1.30 (3H, d, J=6Hz), 1.28 (22H, m), 1.13 (3H, d, J=6Hz), 0.89 (3H, t, J=7Hz), 0.81 (3H, d, J=6.5Hz), 0.80 (3H, d, J=6.5Hz)

(13) Molecular formula and FAB-MS:

C₇₁F₁₁₆N₁₄O₂₃ • HClFAB-MS m/z 1533 (M+H)⁺;

HRFAB-MS m/z 1555.8240

(calcd for C₇₁H₁₁₆N₁₄O₂₃Na: 1555.8235).

(14) Analysis of amino acids:

Thr (4), Gly (1), Ala (1), Val (1), Tyr (1), Orn (1) (as hydrolyzed in 6 N HCl at 110°C for 20 hours)

(15) Ultraviolet absorption spectrum:

Significant peaks are:

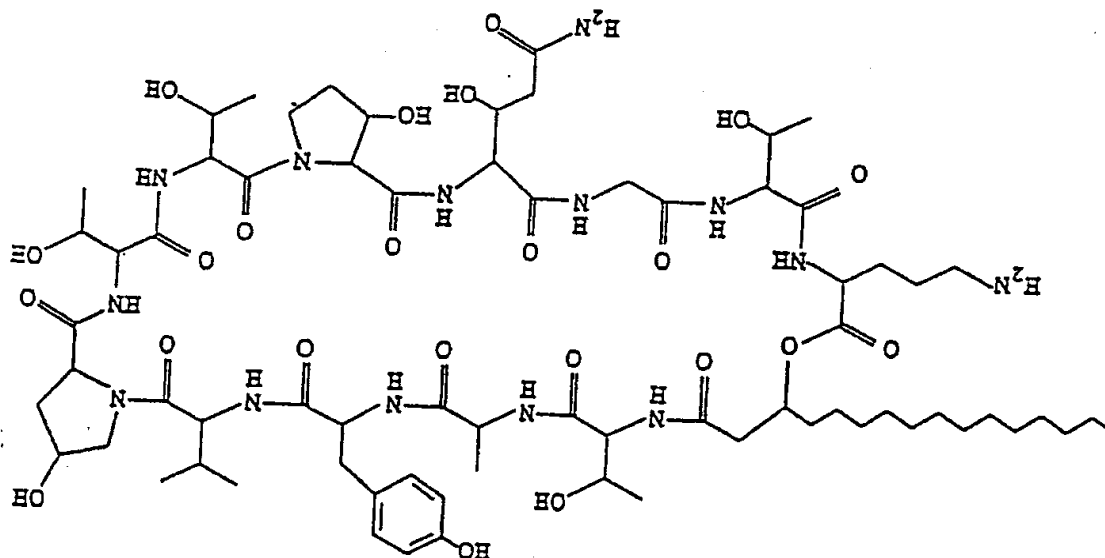
λ_{max} H_2O	225 (sh), 275 (1200), 280 (sh) nm
λ_{max} 0.01N HCl	225 (sh), 275, 280 (sh) nm
λ_{max} 0.01N NaOH	240, 292 nm

3. A method of producing WF11243 substance of claim 1 or 2 and its salts, which comprises culturing WF11243 substance producing microorganism in a medium and collecting the WF11243 substance from the resulting medium.
4. A method according to claim 3, wherein the WF11243 substance producing microorganism is strain FERM-BP3373 and wherein WF11243 substance is defined as in claim 1 or 2.
5. A WF11243 substance producing microorganism according to claim 4, wherein the WF11243 substance producing microorganism is FERM-BP3373 or a mutant or variant thereof.
6. A fungicide comprising WF11243 substance or its salt as an active ingredient, wherein WF11243 substance is defined as in claim 1 or 2.
7. A protozoicide comprising WF11243 substance or its salt as an active ingredient, wherein WF11243 substance is defined as in claim 1 or 2.
8. A protozoicide according to Claim 7, wherein the protozoon is Pneumocystis carinii.

Patentansprüche

- 1. Substanz WF11243 mit der folgenden chemischen Formel (I) oder ihre Salze.**

(I)



2. Substanz WF11243, die als Hydrochlorid die folgenden physikochemischen Eigenschaften aufweist, oder ihre Salze:

(1) Aussehen: weißes Pulver

(2) Schmelzpunkt: 182 bis 187°C

(3) Spezifische Drehung: $[\alpha]_D^{23} +29^\circ$ (c=1,5, Methanol)

(4) Molekülformel: $C_{71}H_{116}N_{14}O_{23} \cdot HCl$

(5) Elementaranalyse:

berechnet (%) für $C_{71}H_{116}N_{14}O_{23} \cdot HCl \cdot 8H_2O$			
	C 49,74,	H 7,82,	N 11,44
gefunden (%):	C 49,65,	H 7,72,	N 11,40

(6) Löslichkeit:

löslich: Methanol, Wasser
etwas löslich: Aceton
unlöslich: n-Hexan

(7) Farbreaktion:

positiv: Ioddampfreaktion, Cersulfatreaktion, Ninhydrinreaktion
negativ: Molish-Reaktion, Ehrlich-Reaktion

(8) Dünnschichtchromatographie (TLC):

Stationäre Phase	Entwicklungslösungsmittel	Rf-Wert
Kieselgel 60 F ₂₅₄ (hergestellt von E. Merck)	n-Butanol/Essigsäure/Wasser (4/1/2)	0,42
RP-18 WF ₂₅₄ S (hergestellt von E. Merck)	45 %iges wäßriges Acetonitril (enthaltend 0,5 % $NH_4H_2PO_4$)	0,18

(9) Infrarotabsorptionsspektrum (FT-IR (KBr)):

Signifikante Peaks sind:

ν_{\max}^{KBr} : 3400, 2920, 1730, 1660, 1650, 1635, 1540, 1520, 1460, 1250, 1090 cm^{-1}

(10) Hochleistungs-Flüssigkeitschromatographie (HPLC):

Säule:

YMC • Pack ODS-AM (AM-303, S-5, 120A, 4,6 mm ID x 250 mm; YMC Co., Ltd.)

Detektion:

210 nm

Entwicklungslösungsmittel:

45 %iges wäßriges Acetonitril (enthaltend 0,5 % $NH_4H_2PO_4$)

Flußrate:

1 ml/min.

Retentionszeit (RT):

10,9 Minuten.

(11) ^{13}C -Kernresonanzspektrum (100 MHz, CD_3OD): Fig. 1 zeigt die Abbildung

δ_{C} : 176.1 (s), 174.6 (s), 174.0 (s), 173.8 (s), 173.4 (s), 172.8 (s), 172.6 (s), 172.5 (s), 172.4 (s), 172.1 (s), 171.9 (s), 171.8 (s), 171.7 (s), 171.2 (s), 156.1 (s), 131.4 (d) $\times 2$, 128.6 (s), 117.9 (d) $\times 2$, 74.2 (d), 72.0 (d), 70.5 (d), 70.1 (d), 70.0 (d), 69.1 (d), 68.8 (d), 68.3 (d), 67.7 (d), 61.4 (d), 60.9 (d), 60.3 (d), 59.8 (d), 58.2 (d), 57.9 (t), 57.6 (d), 57.2 (d), 56.8 (d), 51.7 (d), 50.8 (d), 46.4 (t), 43.1 (t), 42.9 (t), 40.4 (t), 39.9 (t), 38.9 (t), 37.6 (t), 35.8 (t), 34.7 (t), 33.1 (t), 31.6 (d), 30.8 (t), 30.7 (t), 30.7 (t), 30.6 (t), 30.5 (t), 30.4 (t), 30.3 (t), 28.1 (t), 25.9 (t), 24.9 (t), 23.7 (t), 21.2 (q), 20.9 (q), 20.3 (q), 19.2 (q), 18.9 (q), 18.7 (q), 17.2 (q), 14.4 (q).

(12) ^1H -Kernresonanzspektrum (400 MHz, CD_3OD): Fig. 2 zeigt die Abbildung.

δ_{H} : 7.08 (2H, d, $J=8\text{Hz}$), 6.98 (2H, d, $J=8\text{Hz}$), 5.18-5.09 (2H, m), 4.88 (1H, br s), 4.76-4.69 (3H, m), 4.65 (1H, m), 4.48 (1H, m), 4.46 (1H, d, $J=5\text{Hz}$), 4.41 (1H, d, $J=9\text{Hz}$), 4.36-4.27 (3H, m), 4.24-4.13 (6H, m), 3.98 (1H, m), 3.92-3.78 (3H, m), 3.70 (1H, dd, $J=11$ and 4Hz), 3.58 (1H, br d, $J=11\text{Hz}$), 3.00-2.93 (3H, m), 2.80 (1H, dd, $J=13$ and 11Hz), 2.56 (1H, dd, $J=13$ and 11Hz), 2.45 (1H, dd, $J=14$ and 9Hz), 2.37 (1H, br d, $J=13\text{Hz}$), 2.30 (1H, m), 2.21-2.05 (3H, m), 2.03-1.93 (2H, m), 1.89 (1H, m), 1.77 (1H, m), 1.62-1.50 (4H, m), 1.39 (3H, d, $J=6\text{Hz}$), 1.35 (3H, d, $J=6.5\text{Hz}$), 1.35 (3H, d, $J=6.5\text{Hz}$), 1.30 (3H, d, $J=6\text{Hz}$), 1.28 (22H, m), 1.13 (3H, d, $J=6\text{Hz}$), 0.89 (3H, t, $J=7\text{Hz}$), 0.81 (3H, d, $J=6.5\text{Hz}$), 0.80 (3H, d, $J=6.5\text{Hz}$)

(13) Molekülformel und FAB-MS:

$\text{C}_{71}\text{H}_{116}\text{N}_{14}\text{O}_{23} \cdot \text{HCl}$

FAB-MS m/z 1533 ($\text{M}+\text{H}$) $^+$;

HRFAB-MS m/z 1555.8240

(berechnet für $\text{C}_{71}\text{H}_{116}\text{H}_{14}\text{O}_{23}\text{Na}$: 1555.8235)

(14) Analyse der Aminosäuren:

Thr (4), Gly (1), Ala (1), Val (1), Tyr (1), Orn (1) (hydrolysiert in 6 N HCl bei 110°C während 20 Stunden)

(15) Ultraviolett-Absorptionsspektrum:

Signifikante Peaks sind:

$\lambda_{\text{max}}^{\text{H}_2\text{O}}$: 225 (sh), 275 (1200), 280 (sh) nm
 $\lambda_{\text{max}}^{0.01\text{N HCl}}$: 225 (sh), 275, 280 (sh) nm
 $\lambda_{\text{max}}^{0.01\text{N NaOH}}$: 240, 292 nm

3. Verfahren zur Herstellung der Substanz WF11243 nach Anspruch 1 oder 2 oder ihrer Salze, das Kultivieren von Substanz WF11243-produzierenden Mikroorganismen in einem Medium und Isolieren der Substanz WF11243 aus dem resultierenden Medium umfaßt.

4. Verfahren nach Anspruch 3, wobei der die Substanz WF11243 produzierende Mikroorganismus der Stamm FERM-BP3373 ist und wobei die Substanz WF11243 wie in Anspruch 1 oder 2 definiert ist.

5. Die Substanz WF11243 produzierender Mikroorganismus nach Anspruch 4, wobei der die Substanz WF11243 produzierende Mikroorganismus FERM-BP3373 oder eine Mutante oder Variante davon ist.

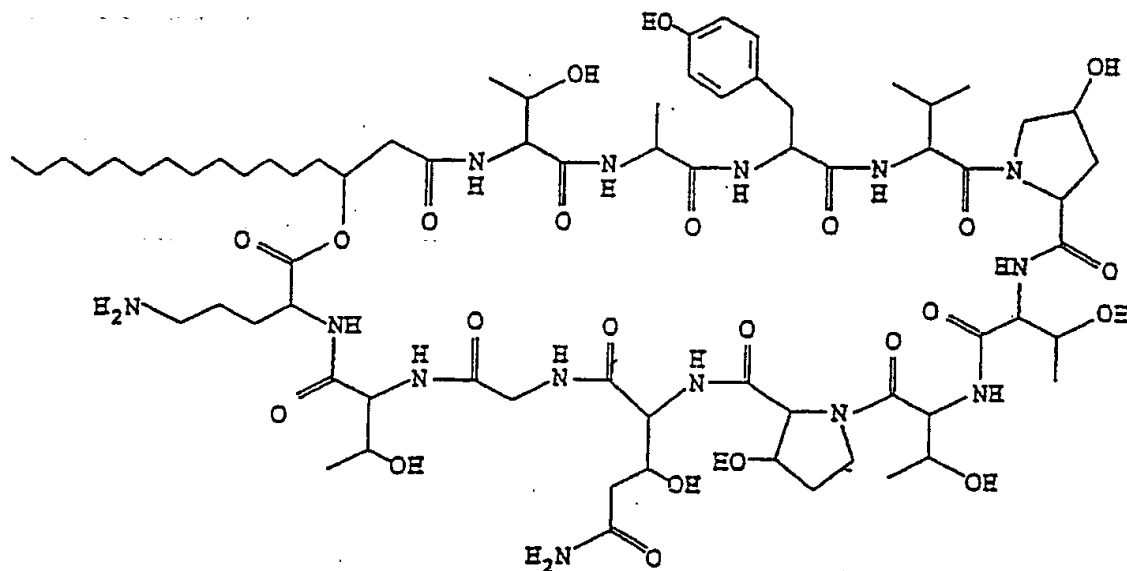
6. Ein Fungizid, umfassend Substanz WF11243 oder ihr Salz als aktiven Bestandteil, wobei die Substanz WF11243 definiert ist wie in Anspruch 1 oder 2.

7. Protozoizid, umfassend die Substanz WF11243 oder ihr Salz als aktiven Bestandteil, wobei die Substanz WF11243 definiert ist wie in Anspruch 1 oder 2.

8. Protozoizid entsprechend Anspruch 7, wobei das Protozon *Pneumocystis carinii* ist.

Revendications

1. Substance WF11243 ayant la formule chimique (I) suivante ou ses sels.



(I)

2. Substance WF11243 ayant, sous la forme de son chlorhydrate, les propriétés physicochimiques suivantes, ou ses sels :

- (1) Aspect :
Poudre blanche.
(2) Point de fusion :
182 à 187°C.
(3) Pouvoir rotatoire spécifique :
 $[\alpha]_D^{23} + 29^\circ$ (c=1,5, méthanol)
(4) Formule brute :
 $C_{71}H_{116}N_{14}O_{23} \cdot HCl$
(5) Composition élémentaire :

Calculé (%) : pour $C_{71}H_{116}N_{14}O_{23} \cdot HCl \cdot 8H_2O$			
	C 49,74,	H 7,82,	N 11,44
Trouvé (%) :	C 49,65,	H 7,72,	N 11,40.

- (6) Solubilité :
soluble dans le méthanol, l'eau
peu soluble dans l'acétone
insoluble dans le n-hexane.

- (7) Réactions colorées :

positives : réaction à la vapeur d'iode, réaction au sulfate cérique, réaction à la ninhydrine.
négatives : réaction de Molish, réaction d'Ehrlich.

- (8) Chromatographie sur couche mince (TLC) :

Phase stationnaire	Solvant de développement	Rf
Gel de silice 60 F ₂₅₄ (fabriqué par E. Merck)	n-butanol/acide acétique/eau (4/1/2)	0,42
RP-18 WF ₂₅₄ S (fabriqué par E. Merck)	acétonitrile aqueux à 45 % (contenant 0,5 % de NH ₄ H ₂ PO ₄)	0,18

(9) Spectre d'absorption infrarouge (FT-IR (KBr)) : Les pics importants sont :

$\nu_{\text{max}}^{\text{KBr}}$: 3400, 2920, 1730, 1660, 1650, 1635, 1540, 1520, 1460, 1250, 1090 cm⁻¹.

(10) Chromatographie liquide à hautes performances (HPLC)

colonne : YMC.remplissage ODS-AM (AM-303, S-5, 1 120A, DI 4,6 mm x 250 mm; Société YMC, Ltd.).

détection : 210 nm. solvant de développement : acétonitrile aqueux à 45 % (contenant 0,5 % de NH₄H₂PO₄).

débit : 1 ml/min

temps de rétention (RT) : 10,9 min.

(11) Spectre de résonance magnétique nucléaire de ¹³C (100 MHz, CD₃OD) :

La Figure 1 représente le spectre.

δ_c : 176,1 (s), 174,6 (s), 174,0 (s), 173,8 (s), 173,4 (s), 172,8 (s), 172,6 (s), 172,5 (s), 172,4 (s), 172,1 (s), 171,9 (s), 171,8 (s), 171,7 (s), 171,2 (s), 156,1 (s), 131,4 (d) x2, 128,6 (s), 117,9 (d) x2, 74,2 (d), 72,0 (d), 70,5 (d), 70,1 (d), 70,0 (d), 69,1 (d), 68,8 (d), 68,3 (d), 67,7 (d), 61,4 (d), 60,9 (d), 60,3 (d), 59,8 (d), 58,2 (d), 57,9 (t), 57,6 (d), 57,2 (d), 56,8 (d), 51,7 (d), 50,8 (d), 46,4 (t), 43,1 (t), 42,9 (t), 40,4 (t), 39,9 (t), 38,9 (t), 37,6 (t), 35,8 (t), 34,7 (t), 33,1 (t), 31,6 (d), 30,8 (t), 30,7 (t), 30,7 (t), 30,7 (t), 30,6 (t), 30,5 (t), 30,4 (t), 30,3 (t), 28,1 (t), 25,9 (t), 24,9 (t), 23,7 (t), 21,2 (q), 20,9 (q), 20,3 (q), 19,2 (q), 18,9 (q), 18,7 (q), 17,2 (q), 14,4 (q).

(12) Spectre de résonance magnétique nucléaire de ¹H (400 MHz, CD₃OD) :

La Figure 2 représente le spectre.

δ_H : 7,08 (2H, d, J=8Hz), 6,98 (2H, d, J=8Hz), 5,18-5,09 (2H, m), 4,88 (1H, s large), 4,76-4,69 (3H, m), 4,65 (1H, m), 4,48 (1H, m), 4,46 (1H, d, J=5Hz), 4,41 (1H, d, J=9Hz), 4,36-4,27 (3H, m), 4,24-4,13 (6H, m), 3,98 (1H, m), 3,92-3,78 (3H, m), 3,70 (1H, dd, J=11 et 4Hz), 3,58 (1H, d large, J=11Hz), 3,00-2,93 (3H, m), 2,80 (1H, dd, J=13 et 11Hz), 2,56 (1H, dd, J=13 et 11Hz), 2,45 (1H, dd, J=14 et 9Hz), 2,37 (1H, d large, J=13Hz), 2,30 (1H, m), 2,21-2,05 (3H, m), 2,03-1,93 (2H, m), 1,89 (1H, m), 1,77 (1H, m), 1,62-1,50 (4H, m), 1,39 (3H, d, J=6Hz), 1,35 (3H, d, J=6,5Hz), 1,35 (3H, d, J=6,5Hz), 1,30 (3H, d, J=6Hz), 1,28 (22H, m), 1,13 (3H, d, J=6Hz), 0,89 (3H, t, J=7Hz), 0,81 (3H, d, J=6,5Hz), 0,80 (3H, d, J=6,5Hz).

(13) Formule brute et FAB-SM :

C₇₁H₁₁₆N₁₄O₂₃.HCl

FAB-SM m/z 1533 (M+H)⁺;

HRFAB-SM m/z 1555,8240

(Calculé pour C₇₁H₁₁₆N₁₄O₂₃Na : 1555,8235).

(14) Composition en aminoacides :

Thr (4), Gly (1), Ala (1), Val (1), Tyr (1), Orn (1) (après hydrolyse dans HCl 6N à 110°C pendant 20 heures).

(15) Spectre d'absorption ultraviolette :

Les pics importants sont :

$\lambda_{\text{max}}^{\text{H}_2\text{O}}$: 225 (épaulement), 275 (1200), 280 (épaulement nm)

$\lambda_{\text{max}}^{\text{HCl 0,01N}}$: 225 (épaulement), 275, 280 (épaulement) nm.

$\lambda_{\text{max}}^{\text{NaOH } 0,01\text{N}}$: 240, 292 nm.

- 5 3. Procédé de production de la substance WF11243 selon les revendications 1 ou 2 et de ses sels, qui comprend le fait de cultiver des microorganismes produisant la substance WF11243 dans un milieu et le fait de recueillir la substance WF11243 dans le milieu obtenu.
- 10 4. Procédé selon la revendication 3, dans lequel le microorganisme produisant la substance WF11243 est la souche FERM-BP3373 et dans lequel la substance WF11243 est définie selon les revendications 1 ou 2.
- 15 5. Microorganisme produisant la substance WF11243 selon la revendication 4, dans lequel le microorganisme produisant la substance WF11243 est FERM-BP3373 ou un mutant ou un variant de celui-ci.
6. Fongicide comprenant la substance WF11243 ou un de ses sels comme ingrédient actif, dans lequel la substance WF11243 est telle que définie dans les revendications 1 ou 2.
7. Protozoocide comprenant la substance WF11243 ou un de ses sels comme ingrédient actif, dans lequel la substance WF11243 est telle que définie dans les revendications 1 ou 2.
- 20 8. Protozoocide selon la revendication 7, dans lequel le protozoaire est *Pneumocystis carinii*.

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FIG. 1

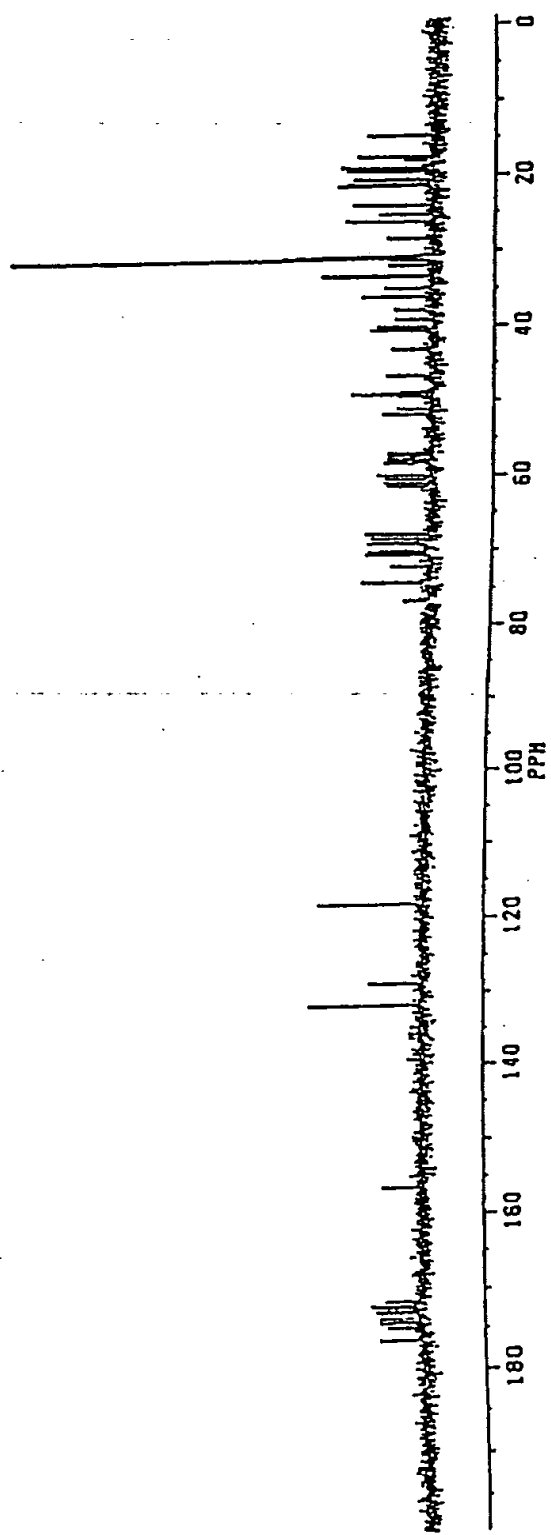


FIG. 2

